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SOMATIC EMBRYOGENESIS IN LEAF CULTURES OF *HEVEA BRASILIENSIS*: EFFECT OF SOURCE PLANT

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Somatic embryogenesis and plant regeneration have been achieved from leaf explants of the clone RRII 105 of *Hevea brasiliensis*. The embryogenic competence of callus induced from leaf explants of the same developmental stage taken from three different sources such as plantlets regenerated through somatic embryogenesis, budded plants grown in polybags and mature budded trees of the clone RRII 105 were examined. Callus induction was obtained in modified MS medium containing 3.6 mM $Ca(NO_3)_2 \cdot 4H_2O$ and 58 mM sucrose, supplemented with phytohormones 2,4-D (5.4 µM), BA (4.4 µM) and NAA (1.08 µM). Proliferated fresh callus was cultured for embryo induction in modified MS basal medium (2.11 mM $Ca(NO_3)_2 \cdot 4H_2O$ and 2.0 mM KH_2PO_4) containing B5 vitamins, amino acids, 220 mM sucrose and phytohormones. Significant difference in rate and the time taken for embryogenic callus initiation was observed. The embryo induction medium containing 1.5 mM $Ca(NO_3)_2 \cdot 4H_2O$ along with 234 mM sucrose and solidified with (0.5%) phytagel was found to be optimal for embryogenic callus initiation. Embryogenic callus could be obtained only from callus induced from *in vitro* - somatic plants and polybag grown - budded plants. Leaves from mature trees produced proliferating callus with little embryogenic competence. Embryo induction was simultaneous with embryogenic callus formation. After the induction of embryogenic callus, the rate of embryogenesis (60%) was similar in the proliferated embryogenic calli derived from leaves of both *in vitro* - derived somatic plants and glass house - grown budded plants. Embryo induction was obtained from proliferated embryogenic calli when the cultures were incubated in dark in modified MS basal medium (2.11 mM $Ca(NO_3)_2 \cdot 4H_2O$ and 2.0 mM KH_2PO_4) containing B5 vitamins, amino acids, organic supplements such as coconut water (5%), malt extract (50 mg/L), casein hydrolysate (300 mg/L), 175 mM sucrose and phytohormones BA (2.2 µM), GA_3 (2.9 µM), Kin (1.25 µM), ABA (0.75 µM) and NAA (0.54 µM). Further embryo maturation and germination, carried out in media standardized earlier, were not affected by the source of explant. The effect of physiological juvenility of source plants and the influence of culture medium on embryogenic tissue initiation are discussed.

Keywords: Carbon source, Embryogenic calli, Gelling agent, *Hevea brasiliensis*, Juvenility of source plants, Somatic embryogenesis.

INTRODUCTION

Somatic embryogenesis is a valuable tool for micropropagation of plant species as well as for genetic manipulation experiments. This regeneration system is generally more difficult with woody plant species. Success of *in vitro* tree regeneration greatly depends on selection of the

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